

## STEROLS IN SURFACE SEDIMENTS OF THE REDANG ISLAND, TERENGGANU

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### ABSTRACT

A total of ten surface sediment samples collected around the Redang Island, Terengganu were used to evaluate sterol variations in the study area. The sediment samples were extracted and analyzed using gas chromatography-mass spectrometer (GC-MS). Generally, the study area is dominated by cholesterol which accounted for 32% of total sterols, followed by phytosterols (27%), marine sterols (20%), fecal sterols (17%) and cholestanol (4%). The sterol source index (SSI) showed low input of phytosterols indicated terrestrial plants source. This might be due to influence of the marine environment of the study area. Furthermore, the sewage contamination index clarified the study area was not contaminated by sewage. The presence of sterols in the study area was derived from various sources which were dominated by inputs from marine sources.

**Key words:** sterol, cholesterol, phytosterol, the sterol source index (SSI)

### INTRODUCTION

Sterols are one of the biolipid compounds that have been successfully used as a marker for the assessment of organic matter input in the aquatic environment. Sterols tend to be adsorbed by sediments which is a typical property of hydrophobic compounds (Froehner *et al.*, 2008) and the compounds tend to stay in the environment for a long period of time since they do not degrade quickly in the environment (Seguel *et al.*, 2001; Carreira *et al.*, 2004). Sterols portrayed specific sources of each of their compound since different group or organisms have different type of sterols (Puglisi *et al.*, 2003; Jardé *et al.*, 2007; Santos *et al.*, 2008). Therefore, these compounds were used to assess the input of organic matter derived from terrestrial and marine sources or anthropogenic sources into the aquatic environment (Seguel *et al.*, 2001; Mudge and Duce, 2005).

This paper aimed to report the investigation on the presence of sterols in surface sediment samples

around the Redang Island, Terengganu and to relate possible sources of each sterol quantified in the area.

### METHODOLOGY

#### Study area

The study area was the Redang Island, Terengganu and the surface sediment samples were collected randomly around the island. Fig. 1 shows the 10 sampling stations in the study area and each coordinate is listed in Table 1. The surface sediment samples were collected using PONAR grab and kept in a glass bottle. Samples were stored in a freezer at <0°C until further analysis.

#### Sterol extraction

The extraction of sterols from sediment sample was carried out according procedures described by Mudge and Norris (1997) and Ali and Mudge (2006). Approximately 30-40 g wet sediment was hydrolyzed with 50 mL of 6% potassium hydroxide in methanol. The sample was refluxed for 4 hours and centrifuged at 2500 r.p.m for 5 minutes. The

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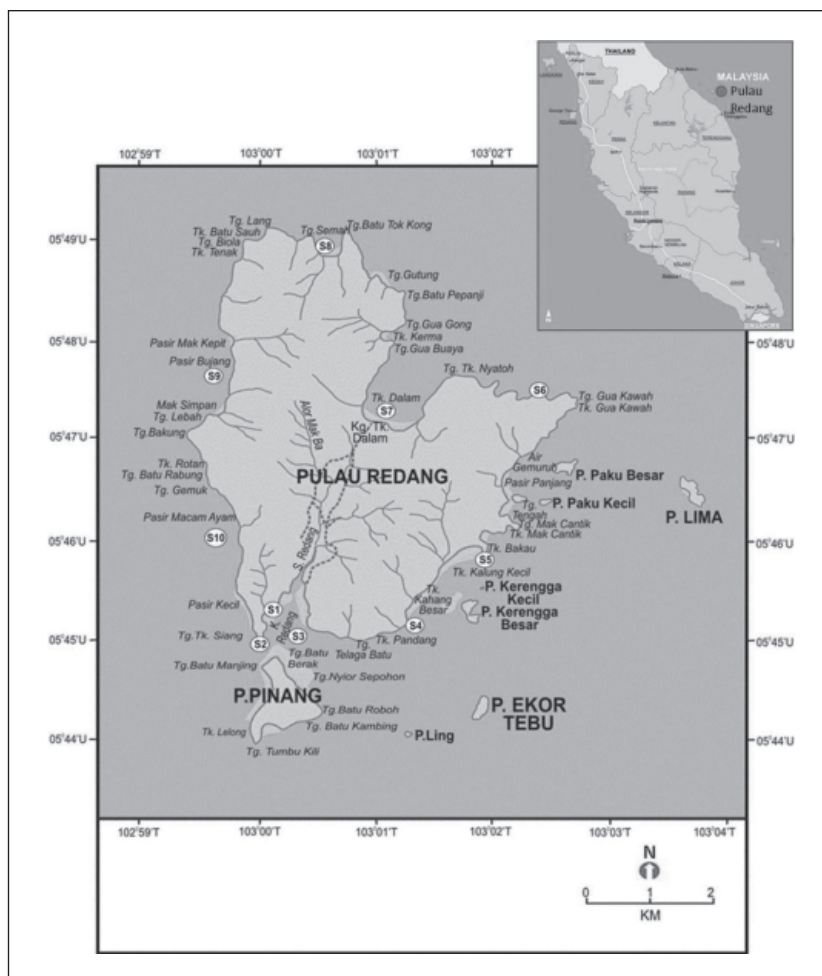


Fig. 1. Study area and sampling stations around Redang Island, Terengganu

**Table 1.** Coordinate of each sampling station at the Redang Island

Sampling station	Coordinate	
	Latitude °N	Longitude °E
S1	05°45'18	103°00'06
S2	05°44'77	102°59'82
S3	05°44'83	103°00'29
S4	05°44'72	103°01'11
S5	05°45'58	103°01'68
S6	05°45'59	103°01'67
S7	05°47'23	103°00'90
S8	05°48'95	103°00'65
S9	05°47'57	102°59'55
S10	05°45'50	102°59'63

supernatant was then funneled into a separating flask.

Non-polar lipids were extracted from the supernatant by liquid-liquid separation. A total of 20 mL of hexane and 10 mL of double distilled water were added to the supernatant. The mixture

was shaken vigorously. After being shaken, the cap of the separating flask was loosened to release the pressure inside. The non-polar fraction was collected and transferred into a florentine flask. The whole procedure was repeated three times to ensure maximum extraction. Samples were evaporated at 40°C using a rotary evaporator, redissolved with 2-3 mL of hexane and then transferred into a 14 mL vial. Anhydrous sodium sulphate was added to remove any water and polar compounds remained in the samples. The remaining solution was filtered through filter paper and blow-dried under oxygen free nitrogen (OFN).

Sample derivatisation was carried out in order to enable the compounds to be analyzed with the gas chromatograph (GC). Approximately 2-3 drops of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) were added to the samples and then heated in a heating block for 10 minutes at 60°C. Finally, they were then evaporated to dryness under OFN and then redissolved in 1 mL of hexane.

A computerized Perkin Elmer gas chromatography-mass spectrometer (GC-MS) model

Clarus 500 was used to analyze the sterols in the samples. The temperature program used started at 80°C, ramped at 15°C min<sup>-1</sup> to 300°C, then at 5°C min<sup>-1</sup> to a maximum of 350°C and hold at final temperature for 10 minutes. Calibration was carried out using a cholesterol-TMS solution in order to quantify the peaks obtained from the analysis. Example of GC chromatogram of sterols is shown in Fig. 2. Meanwhile Fig. 3 shows the mass fragmentation pattern for cholesterol quantified during this analysis. All results are relative to bulk sediment and expressed on a dry-weight basis.

### Quality control

Standard methods and techniques were adopted during this work. In the laboratory, analyses were carried out in Decon-90 washed glassware. The efficiency of the whole extraction process was confirmed by the repeating reflux of some sediment samples, whereby no further sterol compounds could be detected in these later extractions. Blanks and calibration standards were used throughout the GC injections. A blank was injected first and followed by the calibration standard. Five samples were injected afterwards, followed by the blank and

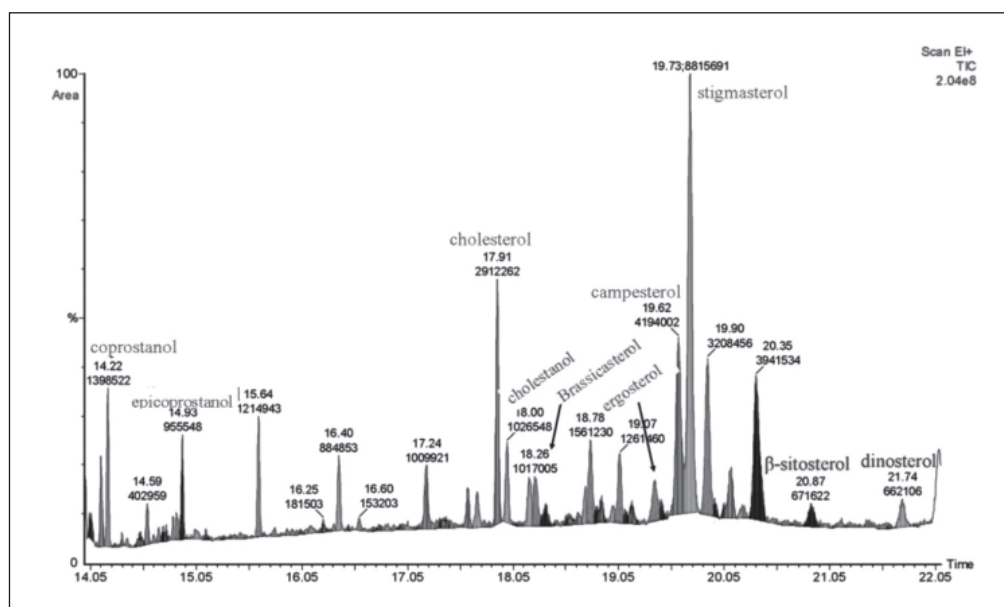


Fig. 2. Chromatogram of sterols obtained from GC-MS analysis

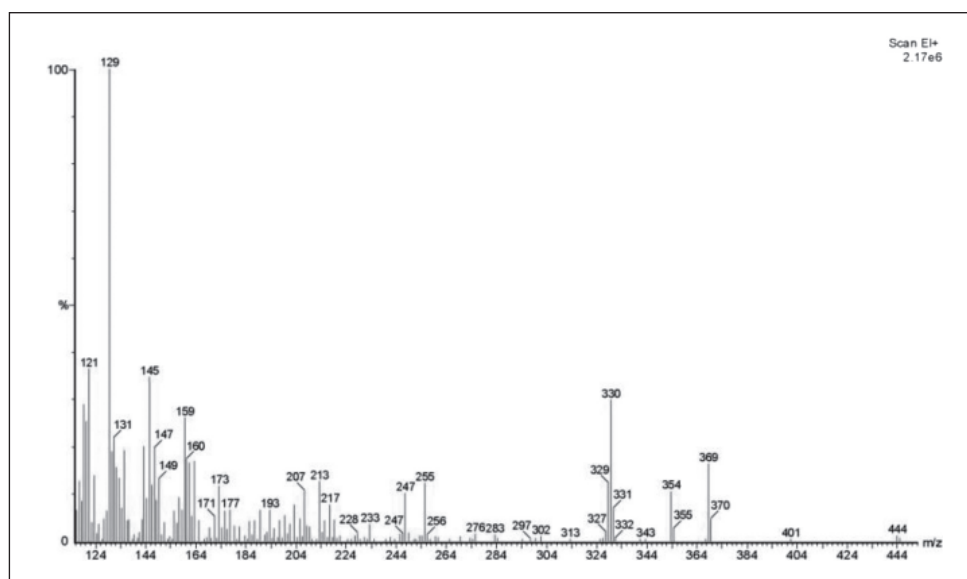


Fig. 3. Mass fragmentation pattern of cholesterol-TMS

calibration standard again. Random samples were extracted three times to test the reproducibility of the extraction. The reproducibility of the extraction was found to be greater than 90%. Procedural blanks were also analyzed and no compounds of interest were measured in any sample. All glassware and Teflon-lined caps used in these analyses were rinsed with organic solvents prior to analysis.

## RESULTS AND DISCUSSION

The results of the sterol analysis based on the dry weight of sediments are listed in Table 2. The data shows that mixtures of sterols from marine, terrestrial, bacterial and fecal sources were identified in all of the sediment samples which represent a total of ten main sterols. Fig. 4 outlines the percentage of each sterol category which including cholesterol, cholestanol, phytosterol, fecal sterol and marin sterol.

Cholesterol, known as the main sterol compound in the environment was the most abundant compound quantified which accounted for 32% of total sterols. Despite the high amount of cholesterol,

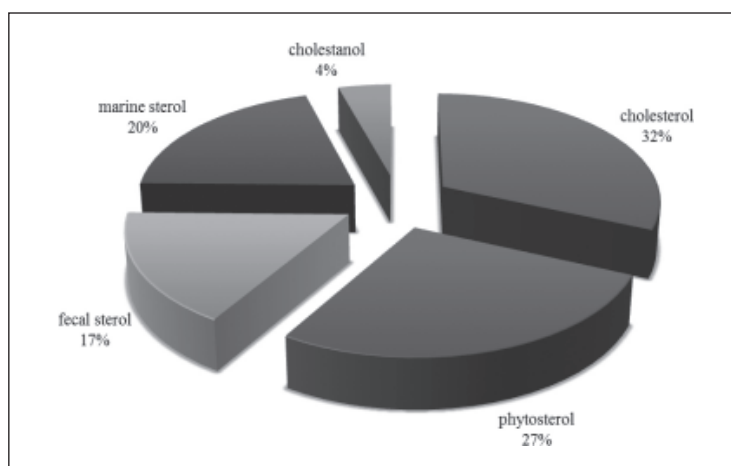
the surface sediments reveal a predominance of phytosterols which accounted for 27% of total sterols. Phytosterols consist of stigmasterol,  $\beta$ -sitosterol and campesterol which are derived from terrestrial higher plants and used to indicate their input into the aquatic system. This was followed by marine sterols (brassicasterol and fucosterol), fecal sterols (coprostanol and epicoprostanol) and cholestanol.

### Cholesterol

Cholesterol was the main sterol in all the sediment samples with concentration ranging from 418.35 to 7058.81 ng/g dry weight sediment. Fig. 5 shows that stations S10 and S7 recorded the highest and the lowest amount of cholesterol of all the sampling stations respectively. All sampling stations investigated have recorded cholesterol as the most abundant compound. This is a typical scenario as the compound is the main sterol found in the aquatic environment (Fernandes *et al.*, 1999; Volkman *et al.*, 2007). As Redang Island is located far from the mainland and surrounded by a marine system, sources of cholesterol might be diatom, microbial community, macrophyte, algae, marine

**Table 2.** The concentrations of sterol in sediment from each sampling stations at Redang Island

Sampling station	Sterol compounds									
	coprostanol	epicoprostanol	cholesterol	cholestanol	brassicasterol	ergosterol	campesterol	stigmasterol	$\beta$ -sitosterol	fucosterol
S1	55.59	1409.31	1790.52	12.66	545.83	15.29	432.73	457.93	960.50	247.94
S2	39.06	1066.39	1945.69	350.88	1446.13	9.43	417.30	832.53	973.93	266.06
S3	17.05	276.11	1445.58	142.97	843.45	25.91	275.39	665.51	803.16	1153.79
S4	51.07	1000.87	3579.4	214.90	1215.33	7.85	306.47	1006.73	861.82	182.84
S5	113.21	2306.04	4240.06	1257.01	1628.44	13.75	599.50	1220.14	1592.33	541.65
S6	69.82	1641.20	3143.68	293.46	1166.44	14.52	513.72	688.01	1087.81	989.18
S7	41.58	268.40	418.45	71.55	204.56	17.12	32.66	116.35	45.39	43.79
S8	106.39	3032.44	3472.89	591.15	1930.05	13.17	634.83	1321.05	1883.51	1009.59
S9	28.87	583.24	1483.50	345.60	1005.49	38.08	325.70	692.16	789.43	127.48
S10	196.31	2726.39	7058.81	748.14	2793.94	70.97	921.10	1788.49	2215.3	1026.38



**Fig. 4.** Percentage of sterol categories identified at the study area

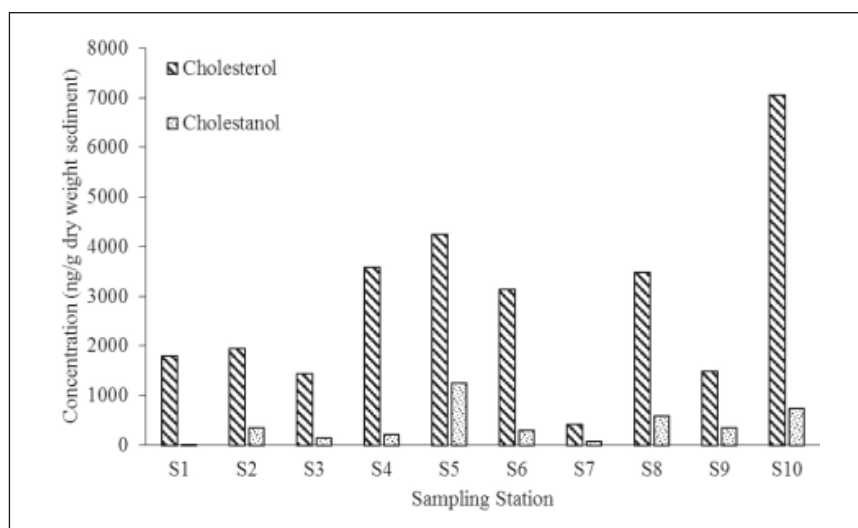


Fig. 5. Concentration of cholesterol and cholestanol at the study area

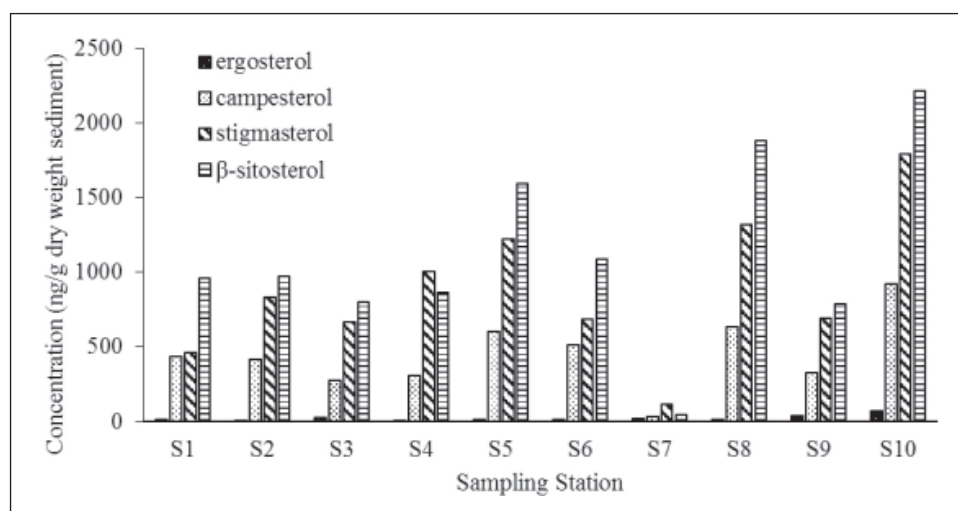


Fig. 6. Concentration of phytosterol compounds at the study area

eustigmatophyte, phytoplankton, zooplankton, benthic organisms and the terrestrial plants of the island (Boot *et al.*, 2006; Hernandez *et al.*, 2008; Reeves and Patton, 2001; Seguel *et al.*, 2001). Cholesterol might also be derived from anthropogenic sources especially sewage matter and agricultural runoff (Mudge *et al.*, 1999; Patton and Reeves, 1999; Reeves and Patton, 2001; Seguel *et al.*, 2001). Thus, due to its various sources, cholesterol as an individual biomarker is limited but has often been used in the ratio form with other sterols (Mudge *et al.*, 1999; Mudge and Duce, 2005).

Cholestanol, one of cholesterol epimers was identified in all sediments samples with a concentration ranging from 71.55 to 1257.01 ng/g dry weight sediment. This compound is formed from bacterial activity towards cholesterol or might be derived from aquatic organisms such as phytoplankton, zooplankton, marine sponges and,

both aquatic and terrestrial plants (Devane *et al.*, 2006; Froehner *et al.*, 2008; Mudge *et al.*, 1999; Seguel *et al.*, 2001).

### Phytosterol

Sterols usually used to indicate the input of terrestrial plants into the aquatic environment are known as phytosterol which consist of three main compounds, namely campesterol, stigmasterol and  $\beta$ -sitosterol. Fig. 6 shows the concentration of each phytosterol at different sampling stations. Ergosterol is another compound categorized together with phytosterols even though the compound is not synthesized by terrestrial plants. Ergosterol is the main sterol biomarker of fungi which involved in the decomposition of terrestrial plants' organic matter. Thus, indirectly ergosterol is the secondary biomarker of terrestrial plants in the aquatic system (Mudge and Norris, 1997; Kodner *et al.*, 2008).

Generally, the major compounds identified were  $\beta$ -sitosterol, followed by stigmasterol and campesterol. Sampling station S10 recorded the highest value of all three compounds compared to other sampling stations. This can be explained by the high density of mangroves along the river mouth area of Sungai Redang heading to the sea. Volkman *et al.* (2007) stated that  $\beta$ -sitosterol is the dominant sterol of mangroves. There are also agricultural activities going on the island that may contributed to the input of phytosterol into the aquatic system. Eventhough terrestrial plants are known as the main sources of phytosterol in the aquatic system, there are also minor contributors of the compounds which are photosynthetic aquatic organisms such as phytoplankton, macrophyte and algae (Giner *et al.*, 2001; Méjanelle and Laureillard, 2008; Santos *et al.*, 2008).

Fig. 7 illustrates the SSI which was used to determine whether terrestrial sterols or marine sterols that dominated the study area, and can be calculated by the ratio of each phytosterol to cholesterol where cholesterol was assumed to be derived from marine sources (Mudge and Norris, 1997). Among those three compounds, SSI also showed that  $\beta$ -sitosterol is the most abundant phytosterol in the study area. However, there is no significant phytosterol distribution which can be concluded based on the results obtained.

### Marine sterols

Marine sterols are usually represented by different types of compounds but the only two compounds detected in the sediments of this study were brassicasterol and fucosterol (Fig. 8).

Brassicasterol concentrations ranged from 204.56 to 2793.94 ng/g dry weight sediment whilst fucosterol concentrations ranged from 43.79 to 1153.79 ng/g dry weight sediment. Overall, brassicasterol concentration was higher than fucosterol concentration at all the sampling stations. Brassicasterol is an indicator of diatom whilst fucosterol is an indicator of seaweed (Mudge *et al.*, 1999; Santos *et al.*, 2008). Higher amounts of brassicasterol might be due to the high density of diatom in the study area compared to seaweed density.

### Fecal sterols

The major fecal sterol, coprostanol was quantified in all the samples with concentrations ranging from 28.87 to 196.31 ng/g dry weight sediment. The epimer or coprostanol, epicoprostanol which is a product formed during the treatment of wastewater and the digestion of sewage sludge was quantified with concentrations ranging from 268.40 to 3032.44 ng/g dry weight sediment. Fig. 9 clearly showed that the amount of epicoprostanol in the study area was much higher than coprostanol.

Coprostanol is produced in the gut of higher animals and the human intestine by the bacterial reduction of cholesterol (McCalley *et al.*, 1980); therefore, it has been widely used as a sewage contamination indicator. However, generally higher epicoprostanol concentration indicated higher amounts of treated sewage matter in the environment. According to Mudge and Lintern (1999), epicoprostanol is only found at low concentrations in areas contaminated by raw domestic sewage.

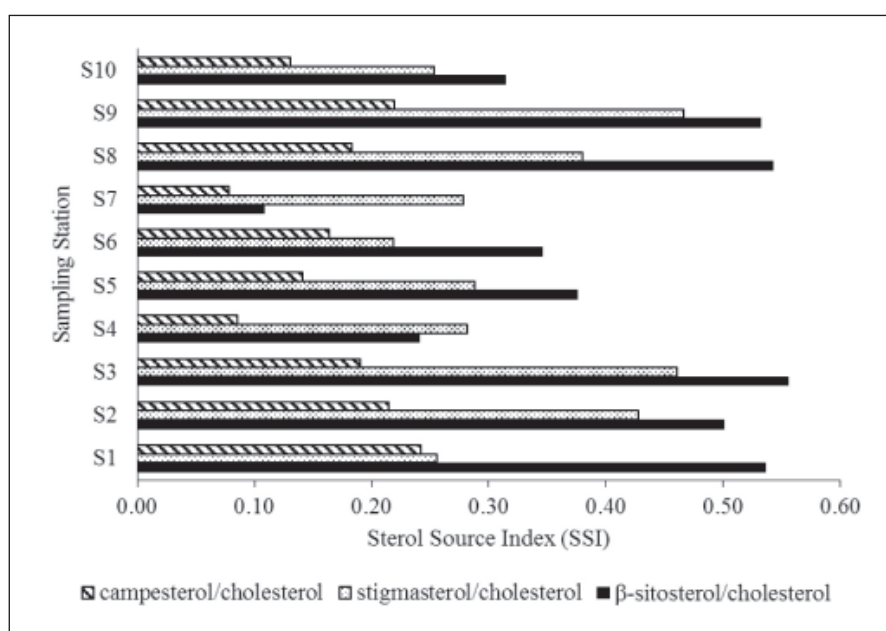


Fig. 7. Sterol ratios for SSI around Redang Island



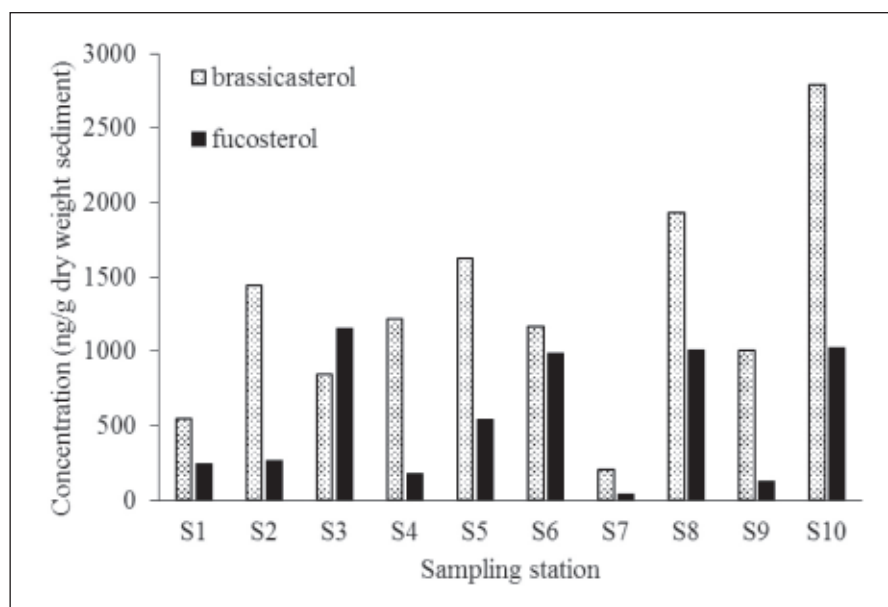


Fig. 8. Concentration of brassicasterol and fucosterol at the study area

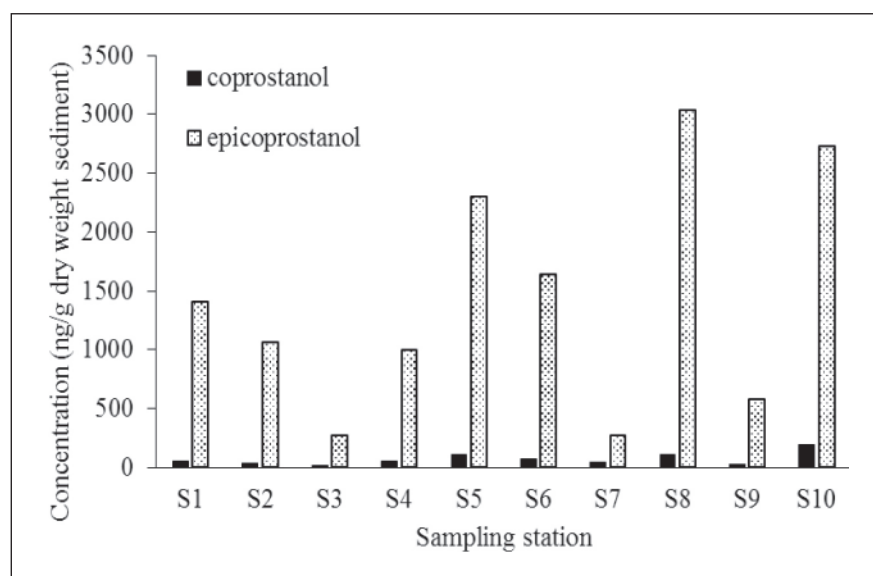
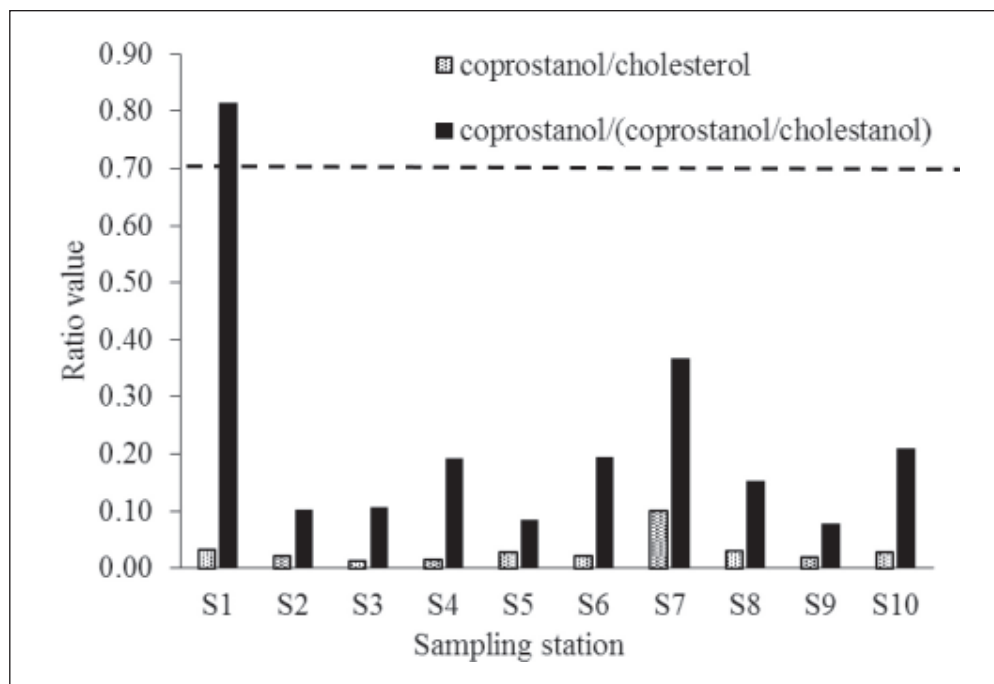


Fig. 9. Concentration of coprostanol and epicoprostanol at the study area

There are two most common indices used to evaluate sewage contamination which are the ratios of coprostanol/cholesterol and coprostanol/(coprostanol+cholestanol) (see Fig. 10). The previous ratio with a value  $>1.0$  is associated with high levels of sewage contamination (Fattore *et al.*, 1996) and the latter ratio with a value  $>0.7$  indicates sewage contamination (Grimalt *et al.*, 1990). None of the samples in this study recorded the ratio  $>1.0$  and only one sample recorded the ratio of  $>0.7$ . Thus, it can be assumed that no significant sewage contamination has occurred in the study area.

## CONCLUSION

This study infers that the sterols quantified from the surface sediment samples of the Redang Island, Terengganu illustrate a mixture of various sources. However, it can be concluded that the study area was dominated by marine organic matter which shown by the high concentration of cholesterol and marine sterols. Furthermore, the minor evaluation on sewage contamination showed that the study area is not contaminated by sewage matter.



**Fig. 10.** Sewage contamination index using coprostanol/cholesterol and coprostanol/(coprostanol + cholesterol) ratios at the study area

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